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Matrix-assisted Laser Desorption/Ionization Mass Spectra Study of Complexed Alkaloid from *Colchicum* Species

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Authors' contributions

This work was carried out in collaboration between all authors. Author JK designed the study, was involved in working out the concept of research, development of a method for the synthesis of colchicine complexes, interpretation of results, writing the main part of the manuscript, literature search and choice. Author GB carried out the analysis and interpretation of MALDI mass spectra. Author GS performed the MALDI measurements, physicochemical consulting. All authors read and approved the final manuscript.

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ABSTRACT

Complexes are biologically useful compounds and they have many biological activities. Colchicine can form stable complexes with alkali metals. Till now colchicine complexes have not been characterised by modern mass spectra methods such as MALDI MS (Matrix-assisted laser desorption/ionization mass spectral). The aim of this study was to check fragmentation pathways of colchicine complexes with alkali metals: Li^+, Na^+, K^+ by MALDI MS mass spectra. The colchicine complexes with Li^+, Na^+, K^+ cations of perchlorates and iodides have been synthesised and studied in details by MALDI MS mass spectra.

Keywords: Colchicine complexes; MALDI MS mass spectra; MALDI TOF MS.

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1. INTRODUCTION

Properly designed metal complexes are interesting materials mainly because of their many applications due to their catalytic or many biological activities as anticancer, antifungal, antiviral and antibacterial. One of the known biologically active compounds is colchicine [1]. Colchicine **1** (Fig. 1.) is a tropolone alkaloid of plant meadow saffron or autumn crocus *Colchicum autumnale* [1]. It shows antimitotic, antifibriotic, anti-inflammatory activity [1] and can efficiently relieve the symptoms during gout attack. More recently it has been introduced in the treatment of familiar Mediterranean fever [2,3]. Moreover, colchicine **1** is a potent anti-mitotic agent and shows carcinogenic activity [4-6]. Similarly to other alkaloids, colchicine **1** can act through blocking or activating of specific receptors or ion channels in living organisms. Its activity depends on the ability of formation of noncovalent complexes with macromolecules such as tubulin in microtubules [7].

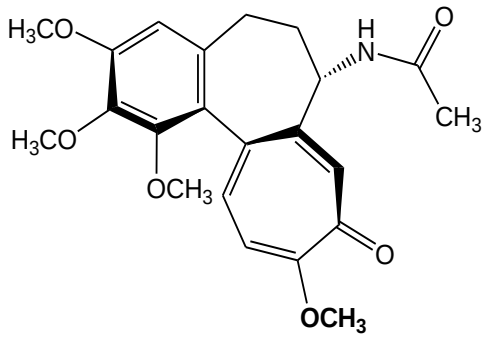

Fig. 1. Structure of colchicine 1

Till now formation of complexes of colchicine and also its derivatives with metal cations have not been studied in details by other spectral methods such as MALDI MS mass spectra [8-10].

Matrix-assisted laser desorption/ionization (MALDI) belongs to the soft ionization techniques in mass spectrometry and therefore it is a method often used to study biological samples and natural compounds. The MALDI matrix protects the sample molecules from direct laser radiation and enables their gentle ionisation and desorption into the gas phase. In this way, complexes of organic compounds with metals and even large supramolecular aggregates, based on weak interactions, such as hydrogen bonds, can be analysed. If the metal in the complex has a characteristic isotope composition, ions containing such an atom or ion are easily recognized in the MS spectrum due to their isotopic pattern. A further advantage of the MALDI technique is the ability to study sparingly soluble compounds, as the sample can be mixed with a matrix in the form of a powder or a suspension, and the desorption of the analyzed ions to the gas phase occurs from the solid phase.

In the previous work, we reported the results of a study on the coordination process of colchicine **1** with iodides and perchlorates of monovalent metal ions (lithium, sodium and potassium salts) [9]. Colchicine complexes with Li^+, Na^+ and K^+ **2-7** have been characterised by spectral methods such as FT IR, UV vis, ESI MS mass spectra, ^{1}H NMR and ^{13}C NMR. Moreover, theoretical studies of the complexation process of colchicine have been made by semiempirical PM5 method and DFT studies [9,10]. Some other theoretical study also has been made on coordination process of colchicine - Na^+ cation [11]. Also for colchiceine (derivative of colchicine) the ability to form stable complexes has been studied [12].

More recently, new series of colchicine complexes with human body fluid ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, CO_3^{2-} and SO_4^{2-} have been obtained and characterized by spectral methods FT IR, ESI MS mass spectra, MALDI MS mass spectra, UV vis, ^{1}H NMR and ^{13}C NMR. Complexation capacity of these complexes has been also studied by DFT theoretical calculation [13].

The aim of this study was examination of colchicine coordinative compound with inorganic salts: perchlorates and iodides (lithium, sodium and potassium) by MALDI MS [9,11]. On the basis of experiments performed and the analysis of MALDI MS spectra studies of these compounds, colchicine was demonstrated to have complexing capacity of the ions mentioned.

2. MATERIALS AND METHODS

Colchicine **1** is commercially available on Sigma-Aldrich. Natural isomer of colchicine (-)-(a*R*, 7*S*) colchicine. Complexes were obtained by dissolving in methanol colchicine and respective salts in ratio 1:1 (Mol:Mol). Salts LiI, NaI, KI, $LiClO_4$, $NaClO_4$, KSCN from Sigma-Aldrich were obtained commercially and used without any purification. Solvents used for the synthesis were purified by standard methods [9]. Colchicine complexes **2-7** were obtained by dissolving of the

respective salts and colchicine in the 1:1 ratio in methanol.

2.1 Measurements

The matrix-assisted laser desorption/ionization measurements, including MS/MS experiments, have been accomplished on Waters Q-TOF Premier instrument, equipped with nitrogen laser MALDI source and MassLynx™ software. MALDI measurements have been performed in the positive ion mode. The MALDI MS experiments have been performed with dithranol as a matrix and argon as colliding neutral gas (at flow rate of 0.5 mL/min). In order to prepare the target spots, the methanolic solution containing matrix (1 µl, concentration 0.5 mol/dm^3) has been deposited on the spot and allowed to dry at room temperature. After a few minutes 1 µL of solution containing colchicine derivative (the concentration about 1 mmol/dm^3) has been placed as a next layer over the dried matrix and left to cocrystallize. The MS/MS experiment with dithranol as a matrix and argon as colliding neutral gas (at flow rate of 0.5 mL/min) has been performed and the collision-induced fragmentation of protonated molecules $[M+H]^+$ has been analyzed, depending on collision energy CE (Table 2). The product ion MS/MS spectra were collected at five collision energy values, i. e. - 20, 30, 40, 50 and 60 eV. It should be noticed that nanospray MS of colchicine and MS^2 of the m/z 400±0.5 ion $[M+H]^+$ have already been reported and representative fragment ions, which originate from m/z 400, are given, i.e. m/z 382, 368, 358, 341, 326, 310, 298 and 282. MALDI MS/MS fully confirms this fragmentation pattern, since the same m/z values appear in collisionally induced dissociation as well as in previously published papers [14].

3. RESULTS AND DISCUSSION

The colchicine complexes with salts ($LiClO_4$, LiI, $NaClO_4$, NaI, KI, KSCN) were obtained as yellow to dark orange crystalline products with very good yields by dissolving of the respective salts and colchicine in the 1:1 ratio [9]. The complexes previously have been studied by spectral analysis: UV-Vis, ^{1}H and ^{13}C NMR, FT IR ESI MS, MS FAB and semiempirical calculation [9].

3.1 MALDI Studies

A six of colchicine complexes with monovalent Li^+, Na^+ and K^+ cations **2-7** has been formed and investigated through MALDI mass spectrometry. The salts: $LiClO_4$, $NaClO_4$, KSCN, LiI, NaI, KI complexes **2-7** have been tested in order to establish the cation and anion importance for complexing process. It should be noticed that nanospray MS of colchicine and MS^2 of the m/z 400±0.5 ion $[\mathbf{1}+H]^+$ have already been reported and representative fragment ions, which originate from m/z 400, are given, i.e. m/z 382, 368, 358, 341, 326, 310, 298 and 282. MALDI MS/MS fully confirms this fragmentation pattern, since the same m/z values appear in regular MALDI spectrum in the negative ion mode induced dissociation as well as in previously published papers [14,15,16].

The obtained colchicine complexes with lithium, sodium and potassium iodides, lithium and sodium perchlorates and potassium thiocyanate [9] were submitted to analysis using MALDI TOF mass spectrometry to check what kind of complexes exist or prevail in the gas phase and how their dissociation proceeds in collision-induced conditions. In the MALDI TOF MS spectra for all tested samples - complexes colchicine-metal cation in a ratio of 1: 1, i. e. $[\mathbf{1} + Li]^+$ for **2** and **3** $[\mathbf{1} + Na]^+$ for **4** and **5** $[\mathbf{1} + K]^+$ for **6** and **7** were observed. The presence of the complexes of stoichiometry 2:1, i.e. $[2\cdot\mathbf{1} + Met]^+$ (where Met is metal ion) was seen as well, but the latter ions have a smaller abundance than $[\mathbf{1} + Na]^+$.

In the MALDI TOF MS spectra for colchicine and its complexes with alkali metal ions (**2**-**7**) with dithranol as a matrix: ions $[\mathbf{1}+Li^+]$, $[\mathbf{1}+Na^+]$ and $[\mathbf{1}+K^+]$ respectively, have been observed, the data (elemental composition and relative abundance) are given in the Table 1.

In the TOF MS LD^+ mass spectra of colchicine complexes with potassium salts some characteristic fragment ions for colchicine itself are observed: m/z= 341.1 and m/z=282.1. Proposed structures of colchicine complexes are given in Fig. 2. Since in MALDI measurements a great excess of matrix (here – dithranol, DIT) in relation to the analyte is used and the matrix is much more prone to deprotonation than colchicine, in the negative ion mode prevail matrix ions (deprotonated dithranol, its dimer, trimer and tetramer, Fig. 4).

As precursors for MSMS experiment, the ions $[\mathbf{1}+Li]^+$ and $[\mathbf{1}+Na]^+$ were chosen. The decay of these ions under collision with argon atoms conditions were examined using collision energy 20, 30, 40, 50 and 60 eV, respectively (Tables 2 and 3, Figs. 4 and 5).

Table 1. MALDI TOF MS spectra of colchicine complexes 2-7

Ion	complex	Elemental composition	m/z	Relative abundance (%)
1+Li$^+$	$C_{22}H_{25}NO_6LiI$ **(2)**	$C_{22}H_{25}NO_6Li^+$	406.1	50
	$C_{22}H_{25}NO_6LiClO_4$**(3)**	$C_{22}H_{25}NO_6Li^+$	406.1	100
1+Na$^+$	$C_{22}H_{25}NO_6NaI$ **(4)**	$C_{22}H_{25}NO_6Na^+$	422.1	85
	$C_{22}H_{25}NO_6NaClO_4$ **(5)**	$C_{22}H_{25}NO_6Na^+$	422.1	70
1+K$^+$	$C_{22}H_{25}NO_6KSCN$ **(7)**	$C_{22}H_{25}NO_6K^+$	438.1	30
	$C_{22}H_{25}NO_6KI$ **(6)**	$C_{22}H_{25}NO_6K^+$	438.1	25
2·**1**+Li$^+$	$C_{22}H_{25}NO_6LiI$ **(2)**	$C_{44}H_{50}N_2O_{12}Li^+$	805.2	10
	$C_{22}H_{25}NO_6LiClO_4$**(3)**	$C_{44}H_{50}N_2O_{12}Li^+$	805.2	1
2·**1**+Na$^+$	$C_{22}H_{25}NO_6NaI$ **(4)**	$C_{44}H_{50}N_2O_{12}Na^+$	821.2	5
	$C_{22}H_{25}NO_6NaClO_4$ **(5)**	$C_{44}H_{50}N_2O_{12}Na^+$	821.2	7
2·**1**+K$^+$	$C_{22}H_{25}NO_6KI$ **(6)**	$C_{44}H_{50}N_2O_{12}K^+$	837.2	10

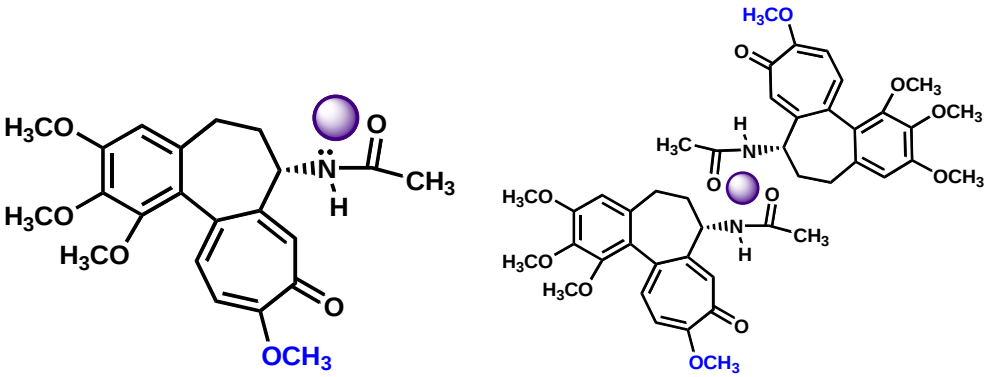

Fig. 2. Proposed structures of complexes formed [Col+M]$^+$ and [2Col+M]$^+$

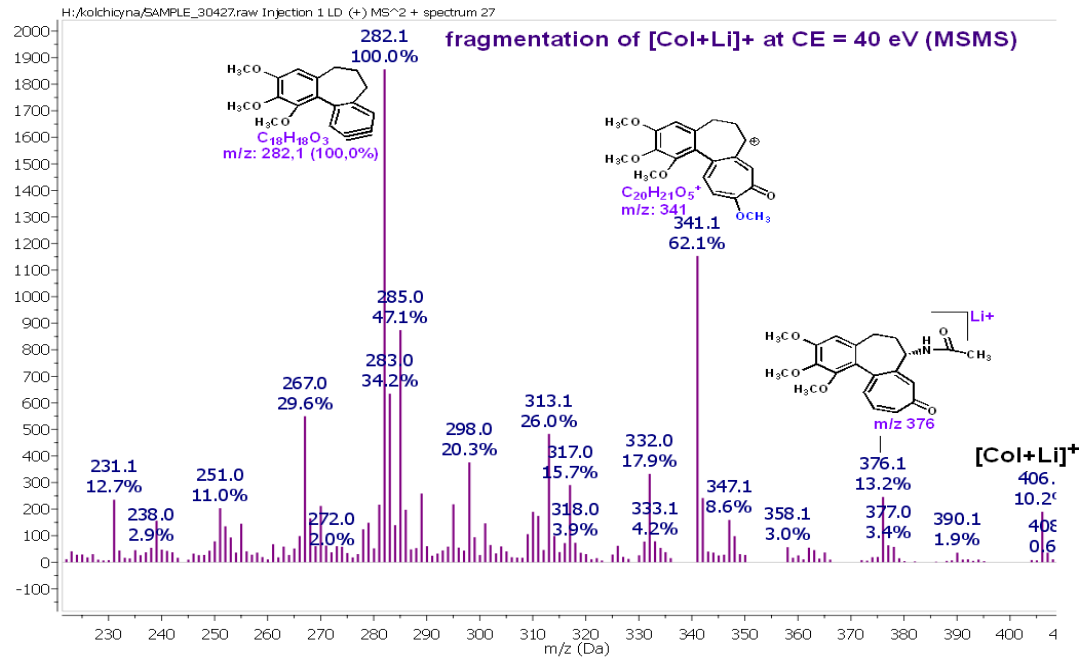

Fig. 3. MALDI (+) TOF MSMS spectrum of colchicine complex with $LiClO_4$ at collision energy CE = 40 eV. Fragmentation of complex ion [Col+Li]$^+$ observed

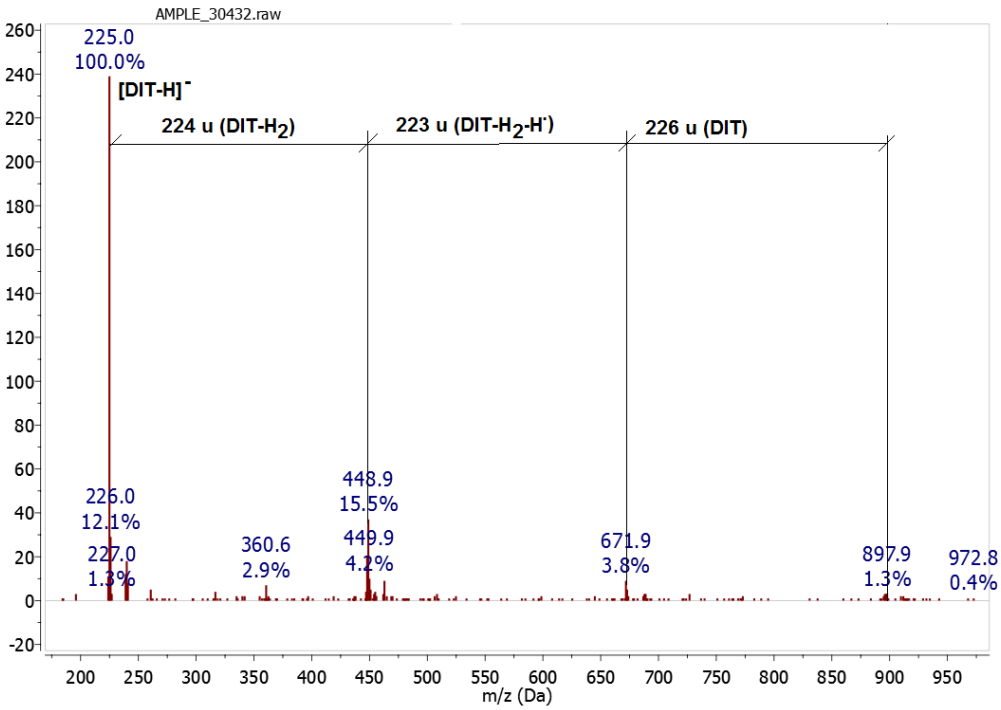

Fig. 4. MALDI TOF mass spectrum of complex 5 (colchicine with $NaClO_4$) in the negative ion mode

Complex ions [**1**+Li]$^+$ and [**1**+Na]$^+$ disintegrate under the influence of collision with argon in a similar manner. The major fragmentation routes include cleavage from the ion [**1**+Me]$^+$ (where Me = Li or Na) of carbon monoxide CO molecule (loss of 28 u) or formaldehyde molecule H_2CO (loss of 30u), where the metal ion remains bound to the colchicine molecule. Occasionally, the methane (CH_4) separation is observed, e.g. the fragmentation reaction 406 → 390 in Fig. 3, but the resulting fragment has a very low intensity (here 1.9%).

Elimination of the acetamide molecule (H_2NCOCH_3) leaving a metal cation associated with the main part of the colchicine backbone is possible and observed, but not preferred (406 → 347, 422 → 363,Tables 2 and 3). Ions at *m/z* 347 for [**1** + Li]$^+$ and 363 for [**1** +Na]$^+$ appear mainly at the collision energy CE=30 eV, and their intensity is low.

The ions, which are formed by removing from the complex ion of an acetamide molecule together with the metal cation and protonation of the neutral molecule formed, are much more intense. This process leads to the formation of an ion at m/z 341 with the composition $C_{20}H_{21}O_5$ and can be described as a substitution of the $H_2NCOCH_3Me^+$ group by a proton.

From the obtained results (Tables 2 and 3) one can conclude that the cations of alkali metals bind quite strongly to the colchicine molecule, since no decomposition with elimination of a metal cation or metal cation exchange on a proton, i.e. [**1**+Met]$^+$+H$^+$ → [**1** + H]$^+$+Met$^+$, had been observed in the MALDI MSMS and no ion at *m*/z 400$_{\bullet}$ (protonated colchicine molecule) had been recorded. On the other hand, a characteristic fragment ion of high abundance in MSMS spectra of the above complex ions is an ion at *m/z* 341.1$_{\bullet}$ indicating a decomposition of the complex with the loss of the NH_2COCH_3 (acetamide group) together with the metal cation, which implies the binding of Li$^+$ and Na$^+$ by the oxygen atom of the amide group.

The other important fragments in the MSMS spectra of the complexes [**1**+ Li]$^+_{\bullet}$, *m/z* 406, are these at m/z 378 and 376 and *m/z* 347 corresponding to the elimination from the complex of masses equal 28u (CO), 30u (CH_2O) and 59u (NH_2COCH_3), while in the case of [**1**+Na]$^+_{\bullet}$, *m/z* 422$_{\bullet}$ respective resulting ions are *m/z* 394 (422-28u), 392 (422-30u) and 363 (422-59u) (Table 3). All of these fragment ions contain in their structure an alkali metal cation, which has not been detached from the colchicine molecular backbone.

Table 2. MALDI MS/MS LD$^+$ spectral data for complexes 2 (1+LiI) and 3 (1+LiClO$_4$) and precursor ion [1+Li]$^+$ *m/z* 406, depending on collision energy (Ce)

m/z	% of relative abundance (% r.a.)									
	Ce = 20		Ce = 30		Ce = 40		Ce = 50		Ce = 60	
	3	2	3	2	3	2	3	2	3	2
406	100	100	100	100	10					
378			9	51						
376					13	13	3			
363				23		10				
347			20	29	9		3			
341		3	84	88	62	39	5			
332			5	13	18	16			5	9
317						32	13			
313			8	10	26	23	3			
298			2		20		16			
295				3			15			
289				9		71	18	46	16	30
285			5		47		26			
282			10	19	100	100	69		16	
267					30	55	100	100	74	65
255						19	34	54	79	
252								54	95	
251					11		35	38		57
239					9		26	46	74	
231			4	6	13	13	11	23		
224							19	38	100	100
209									68	61
207						7	10	38	42	
195							8			57
190							11	15		

Table 3. MS MS LD$^+$ (1 + NaClO$_4$) for the precursor ion [1+Na]$^+$ m/z 422 on different collision energy (Ce)

m/z	% of relative abundance (% r.a.)									
	Ce = 20		Ce = 30		Ce = 40		Ce = 50		Ce = 60	
	4	5	4	5	4	5	4	5	4	5
422	100	100	100	100	9	21				
394			11							
392				11	25	29	2			
363			11	13		7				
341			33	38	41	36				
313			4	6	17	21	3			
298					15					
283									17	
282			6	6	100	100	32	43		
270					18		20			
267					40		100	100	81	67
255							47	36		44
252									54	35
239					9		49	42	75	77
224					3		31	32	100	100
195							14	11	58	42

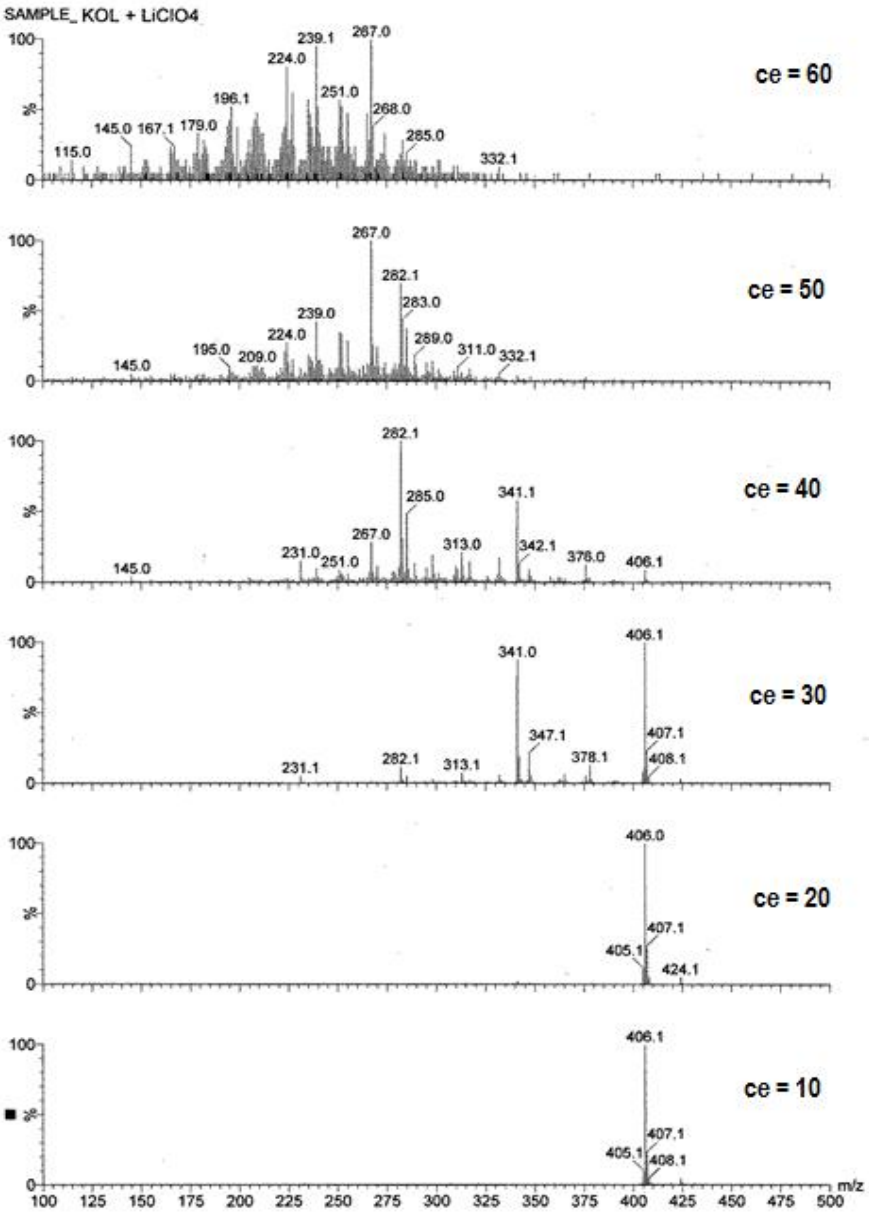

Fig. 5. MALDI TOF MSMS mass spectra of 3 (colchicine complex with $LiClO_4$)

Table 4. MSMS dissociation of the precursor ion at m/z 821, $[2{\cdot}1+Na]^+$, obtained from the colchicine complex with $NaClO_4$ depending on collision energy (Ce)

m/z	Ce	20	30	40	50	60
821.2		100	40	40	23	5
422.1		100	100	100	100	100
408.1					17	63
392.1					30	
363.0					17	12
341.1						48
313.1						12
282.1						30
231.0						22

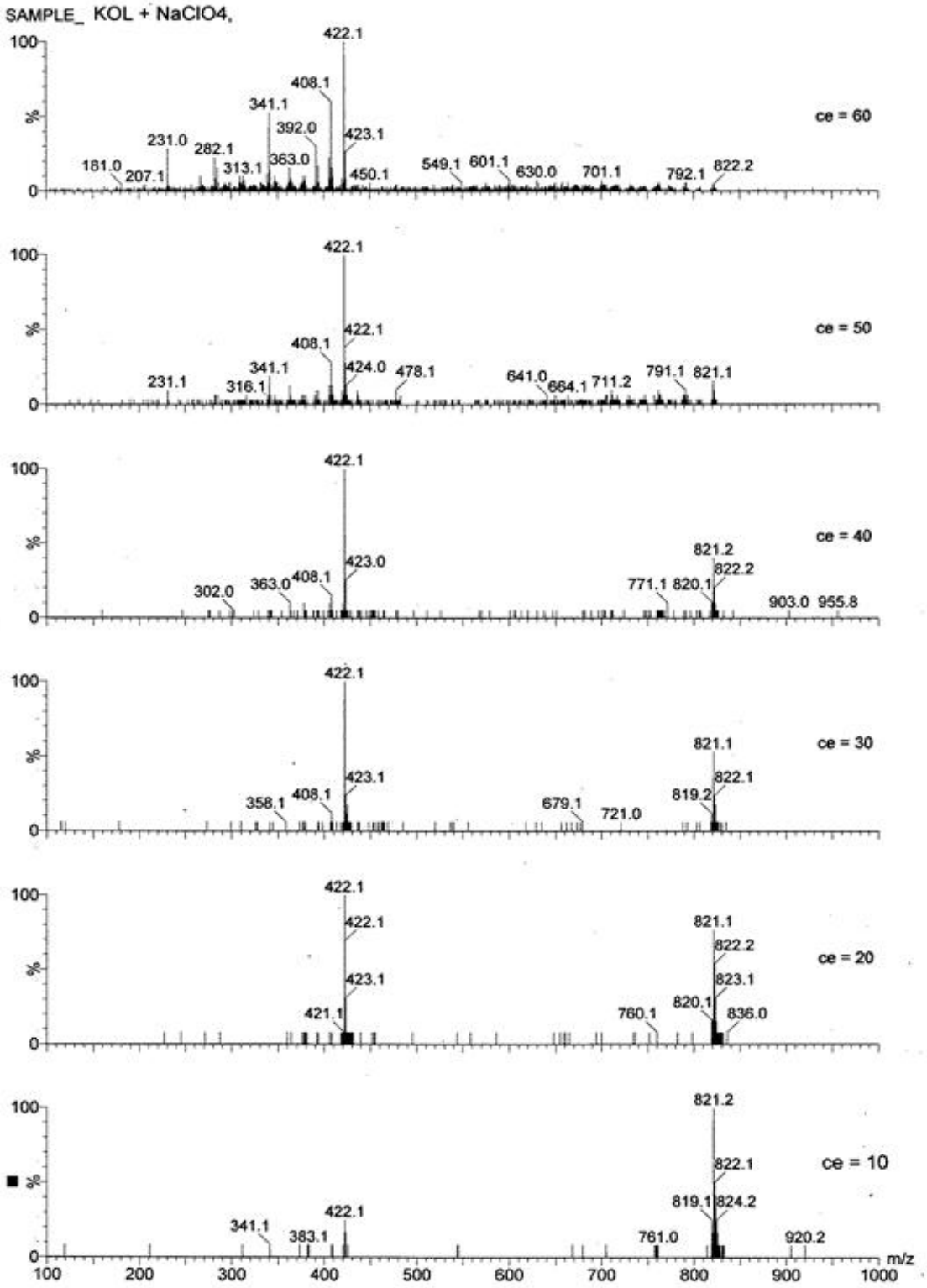

Fig. 6. MALDI TOF MSMS of colchicine complex with $NaClO_4$

The metal cation is detached with the amide group -NH-COCH3 and the ion at m/z 341 is observed.

The further descending masses of the daughter ions differ from m / z 341 by 28 u (CO), 15 u (CH_3), 16 u (CH_4) and 15 u (CH_3), which means

elimination of carbon monoxide and detachment of further methyl groups, either in the form of a radical (· CH_3) or in the form of methane molecule (CH_4). This intense product ions from the precursor [1 + Me]$^+$ can be tentatively pre-composed:

$C_{20}H_{21}O_5$ m/z 341
$C_{19}H_{21}O_4$ m/z 313
$C_{18}H_{18}O_4$ m/z 298
$C_{17}H_{14}O_4$ m/z 282
$C_{16}H_{11}O_4$ m/z 267 or $C_{17}H_{15}O_3$

Common ions with even lower masses are:

$C_{15}H_{11}O_4$ m/z 255
$C_{15}H_8O_4$ m/z 252
$C_{14}H_7O_4$ m/z 239.

It should also be mentioned that in the MSMS spectra of analysed complexes, some characteristic ions for the MS dissociation of [**1**+H]$^+$ ion, are lacking, for example, *m*/*z* 382 (water loss), 368 (MeOH loss), 358 (ketene CH_2=C=O loss) or 310. Frequently observed ion (but with a small abundance) is an ion at *m*/*z* 313. At ce = 40 eV most abundant ion in the MSMS spectra is that at *m*/*z* 282 (100% of relative abundance, $C_{18}H_{18}O_3$), , containing no amide group -NHCOCH$_3$. This ion is known also from MALDI MSMS fragmentation pattern of colchicine and alkylthiocolchicines [14]. For ce = 50 eV, the one of 100% of relative abundance is the ion at *m*/*z* 267, ($C_{17}H_{15}O_3$), [14] and for ce = 60 eV, *m*/*z* 224 (100% r.a.), ion coming from deep decay of colchicine molecule, which is also seen in MALDI TOF MSMS spectra of alkylthiocolchicines at ce = 40 eV [14, Fig. 3].

Dissociation of the dimeric complex [2 · 1 + Na] + (Table 4, Fig. 6) consists mainly in the cleavage of one colchicine molecule and the formation of daughter ion [1 + Na] +. With an increase in collision energy, more and more precursor disappears, and the ion [1 + Na] + has an intensity of 100%. At higher collision energies, few daughter ions are visible, such as formed by the decay of the precursor [1 + Na] +, e.g. m / z 363, 341, 282, 231).

These data indicate that alkali metal ion complexation by colchicine molecule involved is mainly acetamide group located on carbon C7 (see Fig. 1). This conclusion is in agreement with calculations Bodoki et al. [11], who showed theoretically that the most favorable position of the sodium cation is between the oxygen atom of the amide group and the oxygen atom of CH_3O-, located at the C1 carbon atom (see Fig. 1) and that Na+ is located above the methyltropolone ring.

In the case of ions [**1**+K]$^+$ fragmentation in collision conditions is in general very poor, the only daughter ions of significant abundance is the ion at *m*/*z* 149. Similarly proceeded decay of the complexes [**1**+ K]$^+$, formed by colchicine with K_2CO_3 and K_2SO_4 [13], which indicates that the anion has limited effect on the structure and stability of the complex **1**-Met in the gas phase.

4. CONCLUSION

Having already a complex ion detected, then this ion can be isolated and fragmented in collisional dissociation conditions to check how it decomposes i.e. what kind of fragments are formed. The fragmentation pathway may be a characteristic feature of a given compound/complex, on the basis of which it can be distinguished from other compounds. Typically, such fragmentation spectra are placed in databases and then one can compare these spectra of the compound studied with such a base and check whether the fragmentation is identical - if so, it confirms the structure. Series of colchicine complexes with monovalent metal cations of perchlorates and iodides were described by MALDI MS mass spectra. The MALDI experiments suggest that amide group of colchicine plays important role in coordinating the metal ions Li^+, Na^+ and K^+ of the first group of periodic table.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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